***Pseudomonas* CULTURE TECHNIQUE**

Production and Application of Syringomycin E as an Organic Fungicide Seed Protectant against *Pythium* Damping-off

Yukie Kawasaki[1], Claudia Nischwitz[1], Michelle M. Grilley[1], Justin Jones[1,2], Jason D. Brown[2] and Jon Y. Takemoto[1,2]

[1] Department of Biology, 5305 Old Main Hill, Utah State University, Logan, UT 84322, USA
[2] Synthetic Biomanufacturing Institute, 650 East Grand Ave, Utah State University, North Logan, UT 84341, USA

Abstract

Syringomycin E (SRE) is a cyclic lipodepsinonapeptide with potent antifungal activity and is produced by certain strains of *Pseudomonas syringae pv. syringae*. In this study, its potential as an organic-compatible agrofungicide and vegetable seed treatment against the soilborne pathogen *Pythium ultimum var. ultimum* was examined. A variant of *P. syringae pv. syringae* strain B301D with enhanced SRE-producing capabilities was isolated and grown in a bioreactor with SRE yields averaging 50 mg/l in 40 h. SRE was extracted and purified through a large-scale chromatography system using organic-compatible processes and reagents. Organiccompatible and scalably produced SRE is potentially a novel organic fungicide seed protectant.

Winpact Model: FS-02-B10P

Introduction

Strains of *Pseudomonas syringae pv. syringae* are epiphytes and opportunistic pathogens on healthy and susceptible plants, respectively. They produce two classes of non-ribosomal cyclic lipodepsipeptides commonly known as syringomycins and syringopeptins. Syringomycin E (SRE) is an abundant Syringomycin analog with a 3- hydroxydodecanoic acid group attached to a nonapeptide ring (Segre et al. 1989). Antifungal activities of SRE have been reported against many fungal species, including soilborne pathogens such as *Pythium*, *Fusarium* and *Rhizoctonia* (De Lucca et al. 1999).

As a secondary metabolite natural product, SRE is a potential natural fungicide for disease control applications. SRE inhibits oosporic phytopathogens such as *Pythium* sp. making it attractive to explore as a seed protectant against diseases caused by these pathogens.

Pseudomonas syringae pv. syringae B301D is principally used for the production of SRE in potato dextrose broth (PDB) medium (Zhang and Takemoto 1987), syringomycin minimal (SRM) broth (Gross 1985; Mo and Gross 1991) or improved minimal medium (IMM) broth (Surico et al. 1989), and the methods provide SRE levels in milligram quantities after several days of growth, performed typically without agitation.

The goals of this study were to (i) produce scalable amounts of SRE by methods that allow qualification for organic use certification.

Materials and Methods

Medium modification

Modifications of growth medium, SRM (1% w/v glucose, 0.4% w/v histidine, 0.8 mM $MgSO_4$, 1 mM $FeCl_3$ and 0.8 mM potassium phosphate, pH7) (Gross 1985; Mo and Gross 1991), were performed to improve SRE production by *P. syringae pv. syringae*. The antifungal activities of *P. syringae pv. syringae* strain 2B6 culture extracts were examined as a function of different carbon compounds substituted for 1% (w/v) glucose in SRM. Tested were glycerol, ribose, galactose, mannitol, lactic acid, acetate, or succinate, each at 1% (w/v), glucose plus mannitol, each at 1 or 2% (w/v), glucose, 1% (w/v), plus fructose, 0.1% (w/v), and arbutin, 100 µM, and glucose, 1% (w/v), plus fructose, 0.1% (w/v), and salicylate, 100 µM. The inclusion of 1% (w/v) glucose plus 1% (w/v) mannitol gave the highest antifungal activity, and this mannitol-supplemented SRM was named 'improved SRE medium' or 'ISM'. SRE production by *P. syringae pv. syringae* strain G10 was examined with growth in SRM, ISM, SRM plus 100 µM arbutin and 0.1% (w/v) fructose, ISM plus 100 µM arbutin and 0.1% (w/v) fructose, and SRM plus 100 µM salicylate and 0.1% (w/v) fructose in agitated flask cultures.

Scalable SRE production

Pseudomonas syringae pv. syringae strain G10 was initially grown in 500 ml of ISM with rotary shaking (200 rpm) at 28°C for 15 h. The 500 ml culture was used as inoculum for a 10 l ISM culture in a **10 L working volume Winpact Bioreactor and Fermenter System** (**Major Science, Saratoga, CA, USA**) with conditions maintained at pH 6.7, 28°C, 2.5 slpm aeration and 350 rpm stirring. At 40 h after inoculating the bioreactor, SRE was only detected (by HPLC) in the foam that accumulated above the culture medium and not in the culture broth. SRE was collected by siphoning the foam material.

Results

In an agitated and aerated **10 L capacity Winpact bioreactor** and with ISM as growth medium, *P. syringae pv. syringae* strain G10 grew and produced foam on the culture surface. In a typical 40-h bioreactor SRE production run, glucose was consumed while mannitol was not (Fig. 1). The bioreactor culture began to foam on the liquid surface between 15 and 30 h coincident with initial detection of SRE in the culture broth (at 0.036–0.06 mg/l SRE). At 40 h, SRE was detected in the foam above the culture broth but not in the culture broth. The SRE yield averaged ~50 mg/l of culture.

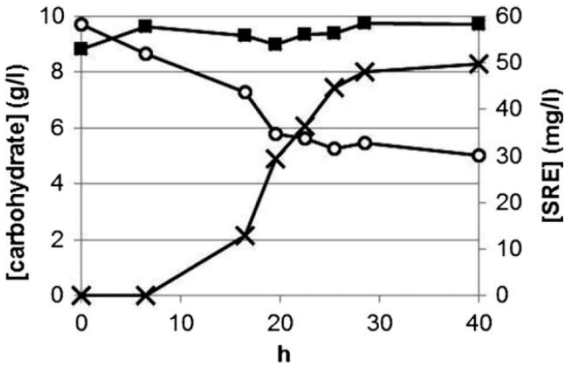

Fig. 1 *Pseudomonas syringae* pv. *syringae* strain G10 growing in ISM in a Winpact Bioreactor and Fermenter system with aeration and agitation produces SRE (×) and consumes glucose (○). Mannitol (■) is not consumed during growth.

References

Yukie Kawasaki, Claudia Nischwitz, Michelle M. Grilley, Justin Jones, Jason D. Brown, Jon Y. Takemoto. (2016) Production and Application of Syringomycin E as an Organic Fungicide Seed Protectant against *Pythium* Damping-off. J Phytopathol 164:801-810

https://onlinelibrary.wiley.com/doi/10.1111/jph.12500

Taiwan Office
No. 156, Sec. 1, Guoji Rd., Taoyuan Dist., Taoyuan City 330041, Taiwan
T/+886-3-3762878
F/+886-3-3761310

US Office
19959 Sea Gull Way
Saratoga, CA 95070
U.S.A.
T/ +1-408-366-9866
F/ +1-408-446-1107

Shanghai Office
Room 612, International business exhibition center,
9300 Hunan Road, Pudong, Shanghai, China
National toll-free No.:400-823-9177
T/ +86-21-50795277
F/ +86-21-50795277

India Office
D.No.7-143, 2nd Floor,
St.No.2,Nagendra Nagar,
Habsiguda, Hyderabad-500007.
India
T/ +91-40-27001515
T/ +91-40-27001586

major science
www.majorsci.com
info@majorsci.com